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Spotlight

Centro de Biologia Molecular "Severo Ochoa": A Center for Basic Research into Alzheimer's Disease

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Abstract. One important aspect of studies carried out at the Center for Molecular Biology "Severo Ochoa" is focused on basic aspects of Alzheimer's disease, mainly the search for suitable therapeutic targets for this disorder. Several groups at the Center are involved in these studies, and, in this spotlight, the work they are carrying out will be described.

INTRODUCTION

Alzheimer's disease (AD) is currently the most prevalent neurodegenerative disorder in humans, affecting nearly 30 million people worldwide [1]. About 1.1% of the total population of developed countries suffer from the disease, and while no cases are found in individuals younger than 20 years of age, there is an incidence of about 30% in people aged 85 years or older [2]. Thus, aging is the most important risk factor for AD [3]. Since the average age of the population continues to increase, the number of patients with AD is expected to rise exponentially, and it is projected that there will be about 110 million people affected with the disease by 2050 [4]. Many laboratories around the world are looking for the means to prevent this devastating disease, and indeed, this is the objective of several groups working at the Center for Molecular Biology" Severo Ochoa".

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THE CENTER FOR MOLECULAR BIOLOGY "SEVERO OCHOA"

The Center is a multidisciplinary Institution supported by the Universidad Autónoma of Madrid (UAM) and the Consejo Superior de Investigaciones Científicas (CSIC-Spanish Higher Research Council). The Center was founded in 1975 based on the proposal of Prof. Severo Ochoa (Nobel Prize winner) with the help of

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Prof. Federico Mayor (former Director of UNESCO) and Prof. Eladio Viñuela. The Center was officially opened by the King and Queen of Spain and initially contained four departments headed by Drs. Mayor, Viñuela/Salas, Vazquez, and García Bellido. One of these, the Neurochemistry Department, was the precursor of our actual Department of Neurosciences. Since then, the research areas at the Center have expanded to incorporate groups working in several other fields such as developmental biology, virology, immunology, molecular microbiology, cell biology, and the regulation of gene expression. In addition, the center now offers a range of scientific services and shared facilitates to support the day-to-day research activities.

The groups that belong to the Department of Neurochemistry are working on many different areas of cell and molecular neurobiology, involving studies into neurotransmitters, transporters, neural stem cells, neuron aging, neuron repair, or neurological signaling. In addition, a large number of groups in the Department are working on neurodegeneration, spinocerebellar ataxias, Huntington's disease, and AD.

In this article, we shall focus on the work carried out by the different groups that focus on distinct aspects of AD.

IDENTIFICATION OF ALZHEIMER'S DISEASE GENES BY FUNCTIONAL GENOMICS

M.J. Bullido/F. Valdivieso

Summary

This group has developed neuronal cell models of human pathogenic processes in order to search for novel markers and therapeutic targets for the genetic and sporadic forms of AD. Gene expression and gene silencing studies are carried out in models, as well as genetic association studies in case control samples that are obtained through a close collaboration with clinical researchers at the University Hospital "la Paz". The group is part of research networks like the CIBERNED and the recently created Biomedical Research Institute of "la Paz" Hospital (IDIPAZ). While the models have been developed independently from other groups, like the genomic and gene silencing experiments, the genetic association studies are made possible by an interaction with the clinical group, which is reinforced by collaborative projects within the CIBERNED network.

For the past few years, the group's work has focused on mouse models of infection with herpes simplex virus 1 (HSV1), in which the involvement of APOE in HSV1 neuro-invasiveness was described, as well as the latency and maternal transmission to the progeny [5–8]. In addition, a genetic association of sporadic AD with genes relevant to HSV1 infection in humans (TAP2, EIF2AK2, GNB3/ADRB1) was also reported [9–11].

Human neuronal cell models of oxidative stress, endoplasmic reticulum stress, amyloid-β protein precursor (AβPP) mutations, and HSV1 infection have been developed and their analyses with genomic tools is underway.

The group's most recent findings include: the demonstration that oxidative stress specifically modulates cholesterol metabolism in neuroblastoma cells; that the silencing of HMGCR, a key enzyme in cholesterol biosynthesis, inhibits the oxidative stress-induced apoptosis; and that HMGCR is genetically associated with sporadic AD [12]. In conjunction, these data reveal a connection between oxidative stress, cholesterol, and apoptosis in the pathogenesis of AD. Further analyses of this model has shown that oxidative stress influences the metabolism/processing of AβPP, modulating the production of the amino terminal proteolytic fragments (soluble extracellular AβPP), and of the intracellular carboxyl terminal αCTF (non-amyloidogenic) and βCTF (amyloidogenic) fragments.

In the cell models of familial AD (neuroblastoma cells stably expressing "wild type" or mutant AβPP), it has been possible to show that mutations sensitize the cells to the damage induced by oxidative stress, and that the AβPP processing induced by oxidative stress depends on the form of AβPP carried by the cells ("wild type" or mutant).

Regarding the neuronal cell model of viral infection, this group found that HSV1 induces an accumulation of autophagic vesicles in the later stages of infection, due to a defect in the fusion of these vesicles with lysosomes. The defect in autophagic degradation, together with the inhibition of Aβ peptide secretion and the increase of γ-secretase activity also induced by HSV1, produces a marked accumulation of intracellular Aβ in the autophagic vesicles of infected cells. Moreover, the effects of HSV1 on autophagy and on AβPP processing are exacerbated in the presence of oxidative stress. In conclusion, HSV1 can produce the characteristic pathological hallmarks of AD neurodegeneration and indeed, the deregulation of both processes induced by HSV1 could contribute to the accumulation of Aβ characteristic of AD.

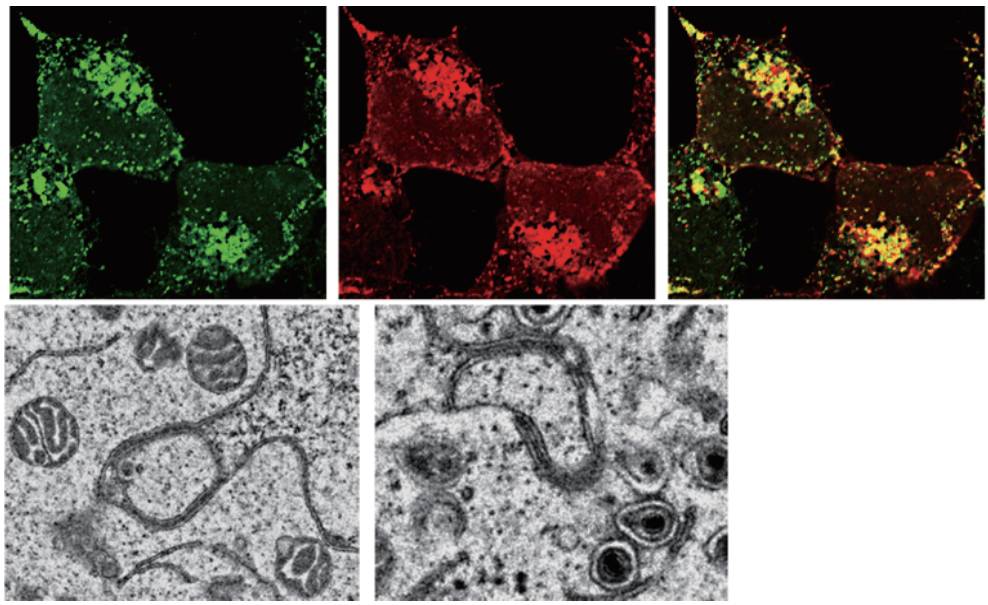

Fig. 1. Confocal and electronic micrographs of HSV1 infected neuroblastoma cells, illustrating the onset of autophagy in the infected cells (upper panel, co-localization of HSV1 with LC3; lower panel, multimembrane autophagic vesicles).

In addition, this group is also involved in collaborative genetic association studies, like the European Consortium that has reported one of the biggest AD genome-wide association study (GWAS) performed to date [13]. Moreover, the group continues to consolidate its collaboration with other groups participating in CIBERNED that are active in this area, collaborations that are proving to be very interesting and fruitful [14–16].

In the near future, the goal of this group is to focus on the genomic analysis of the cell models of AβPP mutations and HSV1 infection, together with studies in humans: genetic association and "in situ" searches for infectious agents, mainly using samples provided by Spanish Brain Banks associated to CIBERNED.

Collaborations in Spain

The group collaborate with others involved in the search of AD susceptibility genes, like Drs. Ana Frank (Hospital "la Paz". Madrid), Elisabet Vilella and Marcel Rosich (University Rovira i Virgili and Hospital Pere Mata. Reus), Onofre Combarros (Hospital "Marqués de Valdecilla". Santander), Jordi Clarimón (Hospital "Sant Pau". Barcelona), Eva Carro (Hospital "12 de Octubre". Madrid), Miguel Calero (ISCIII. Madrid) and Pau Pastor (CIMA. Navarra), and also with Drs Isidro Ferrer (Human Brain Bank, Neuropathology Institutute of the Bellvitge Hospital, Barcelona) and Alberto Rábano (Human Brain Bank, Fundacion CIEN, Madrid).

Collaboration in Europe

European Consortium for Alzheimer's Genetic Research. Coordinated by the Institut Pasteur de Lille (JC Lambert/P Amouyel).

Educational activities

- Professors Valdivieso and Bullido are regularly involved in University teaching activities, giving lectures on the graduate and post-graduate (Master) courses in Biochemistry and Molecular Biology.
- They also lectures in specialist courses in the fields of genomics and neurodegenerative diseases (10-15 lectures/year).
- They participate in graduate and master's practical classes (1/2 students per academic year).
- In the last five years, the group has presented eight doctoral theses.

GSK3 AND ALZHEIMER'S DISEASE

F. Hernandez

Glycogen synthase kinase 3 (GSK3) is a protein kinase for which two isoforms exist, GSK3α and GSK3β. Deregulation of neuronal GSK3 activity has been postulated as a key feature in AD, mainly because GSK3 (mainly GSK3β) interacts with many of the cellular components associated with AD neuropathology, such

as AβPP, presenilins, and the tau protein (the main component of neurofibrillary tangles) [17]. A transgenic mouse with forebrain overexpression of GSK3β (GSK3 mice) has been generated, and it recapitulates many aspects of AD neuropathology, such as tau hyperphosphorylation, reactive astrocytosis, and neuronal death [18], as well as spatial learning deficit [19]. Since transgene expression in these GSK3 mice is conditional using the Tet-off system, it is possible to test whether the biochemical, histopathological, and behavioral consequences of elevated GSK-3 expression can be reverted when normal GSK-3 activity is restored (as an ideal inhibitor might do). In fact, shutdown of GSK3 overexpression led to normal GSK3 activity, normal phospho-tau levels, diminished neuronal death, and suppression of the cognitive deficit, further supporting the potential use of GSK3 inhibitors in the treatment of AD [20].

To further study the involvement of GSK3 in AD, transgenic mice that overexpress GSK3β and FTDP-17 tau have been crossed [21]. The CA1 hippocampal neurons in this AD animal model, termed GSK3/VLW, accumulate hyperphosphorylated tau in the region where the pattern of expression of both transgenes overlaps. Tau filaments with a paired helical filament (PHF)-like structure were found in GSK3/VLW mice but not in single transgenic mice expressing only GSK3β or FTDP-17 tau, and the formation of PHF-like filaments in GSK3/VLW mice was accompanied by thioflavin-S staining. All these data suggest that there is a synergistic contribution of both types of tau modification, hyperphosphorylation and missense mutations, that induces aberrant tau aggregation. This animal model has been used to study the effects of lithium, a GSK3 inhibitor used to treat affective disorders and with well documented effects in humans [22]. In this model, two questions were assessed: first, whether chronic lithium treatment prevents the formation of the aberrant tau aggregates generated by overexpression of FTDP-17 tau and GSK3β; and second, whether lithium can return the tau aggregates and neurofibrillary tangles generated to their former state in aged animals. The results indicated that lithium could prevent the development of the tau pathology when administered early in disease progression. Also, even when administered at later stages of the disease, lithium was able to reduce tau hyperphosphorylation although it could not reverse tau aggregation. These data offer encouragement to carry out studies based on novel GSK3 inhibitors as new pharmacological treatments of these kinds of neurodegenerative disorders [23].

A characteristic of the transgenic models overexpressing GSK3 is a reduced and atrophied dentate gyrus [21]. In collaboration with Dr. Verdugo (Valencia, Spain), it was recently demonstrated that these alterations might be due to a failure of the proliferation and maturation of stem cells in the adult dentate gyrus, which represents a novel view of AD progression [24]. Thus, GSK3β overexpression not only produces a reduction in adult neural stem cells in the sub-granular zone, but also in their proliferative capacity. Transgenic mice develop fewer neurogenic niches and there is less proliferation in these niches, leading to their almost complete depletion with age, phenomena which could play a role AD in humans.

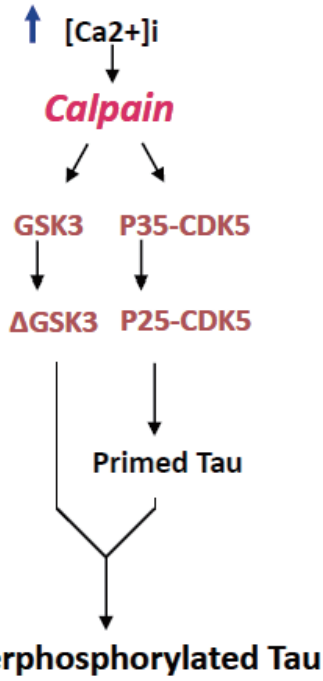

Fig. 2. Altered calcium homeostasis in AD may contribute to the physiological, and eventually to the neuropathological activation of the calpain/GSK3/CDK5 pathway, and to tau hyperphosphorylation.

During these studies with animal models, additional bands of lower apparent molecular weight were consistently found in overexposed immunoblots probed with some anti-GSK3 antibodies, suggesting the presence of specific proteolytic fragments. The effects of some proteases were examined and the involvement of calpain in cleaving GSK3 was demonstrated [25]. Moreover, it was shown that GSK3β is truncated by calpain *in vitro*, while GSK3α is not such a good substrate. The N-terminus of the kinase is the region cleaved by calpain and this cleavage of GSK3 was shown to take place in cortical neurons after glutamatergic stimulation. Interestingly, memantine, a non-competitive NM-

DA receptor that has been approved for the treatment of moderate to severe AD, can inhibit the truncation of GSK3 induced by NMDA in primary cultures of cortical neurons in a dose-dependent manner [26]. Together, these data demonstrate a new means of regulating GSK3 (Fig. 2) with important implications for its physiological and pathological activity.

In the last five years (2006–2010), the work carried out by this group has given rise to three theses and thirty eight scientific manuscripts. The group has received grants from the Fundación La Caixa, Comunidad de Madrid, and Fundación CIEN.

MOLECULAR MECHANISM OF NEURODEGENERATION AND REGENERATION

F. Wandosell/JJ Garrido

Summary

The efforts of this group are devoted to the analysis of the molecular mechanisms triggered by neurodegenerative processes. The aim of these studies is to determine the key signals that regulate cell morphogenesis and how the pathways they modulate are affected in pathological situations such as AD or ischemia. In addition, possible regenerative mechanisms are investigated.

Analyzing cell and animal models of neurodegeneration, it has been possible to show that increases in GSK3 activity correlate with neuronal degeneration, and in some cases with neuronal death. In some of these models, the PI3K-Akt-GSK3 pathway was seen to be deregulated, which was at least in part responsible for the process of neurodegeneration. The group is now deeply committed to understanding how the activity of GSK3 is regulated. Complementing these studies are the efforts to try to determine the role of some neuroprotective hormones, such as estradiol. Recent data demonstrates that estradiol inhibits GSK3 in a similar fashion to IGF-1 [27,28]. Hence, it is important to identify elements implicated in this neuroprotection, and to address whether the activation of "hormonal neuroprotection" may influence the progress of AD or ischemia in the models available.

In addition efforts are being made to understand the key signals that regulate neuronal morphogenesis, particularly having demonstrated that GSK3 is essential to determine, and to maintain, certain properties of axons in models of axonal polarity. More elements are now being analyzed, either upstream or downstream of GSK3, as key cues in axonogenesis. Thus, the aim is to more fully define this cellular compartment, which is critical to neuronal function.

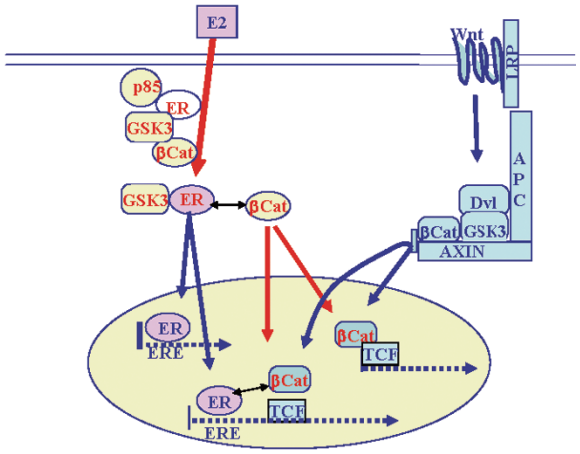

Fig. 3. The scheme represents a summary of the recent results from this group (bold red lines and red letters), indicating a new pathway in which β-catenin may be regulated by either the Wnt pathway and/or estradiol signaling.

Collaborations in Spain

- Collaboration with Dr. I Anton (CNB, Madrid), "Role of actin binding protein in neuronal morphogenesis".
- Collaboration with Dr. J.M. Torres (CISA, Madrid) and Dr. A. del Rio (UB, Barcelona) on prion neurodegeneration.
- R& D Collaboration agreement with FAES FARMA and NOSCIRA.

Collaborations in Europe

Seventh Framework Program NMP: Nanosciences, Nanotechnologies, Materials and new Production Technologies: "Nanoparticles for Therapy and Diagnosis of Alzheimer's Disease"- (NAD). Grant ref: CP-IP 212043-2 NAD.

Educational activities

- Masters in "Molecular Biomedicine", Unv. Autonoma Madrid-(UAM, BMM6)- Coord. Dr. J. Díaz-Nido and Dr. F. Wandosell.
- Masters in "Cell and Molecular Biology", Unv. Autonoma Madrid-(UAM, BM9) Coord.- Dr. I. Antón and Dr. M. Cervera.

CONDITIONAL MOUSE MODELS TO STUDY CENTRAL NERVOUS SYSTEM DISORDERS

J.J. Lucas

Dr. Jose J. Lucas and his research team focus their efforts on the generation and characterization of transgenic mouse lines to study neurodegenerative diseases and other central nervous system conditions. Dr. Lucas was a pioneer in generating conditional transgenic mouse models to explore the reversibility of neurodegenerative diseases. The inducible mouse model of Huntington's disease was the first example of such an approach, and with which the reversibility of the neurological phenotype of the disease was demonstrated through the *in vivo* clearance of the characteristic intraneuronal inclusion bodies [29]. Such reversal also takes place in advanced stages of the disease when the mice have already experienced significant loss of striatal neurons [30]. These results provided the proof of concept for the currently ongoing initiatives of mutant huntingtin silencing as a therapeutic strategy for Huntington's disease and other poly-glutamine disorders [31].

Transgenic mice that conditionally express GSK3 have been generated in collaboration with Drs. Avila and Hernández. Given the aforementioned implications of GSK3 in the etiology of AD, these mice are considered to be an interesting mouse model of AD that recapitulates tau hyperphosphorylation and neuronal death [18], as well as the learning deficits associated with AD [19]. These mice are also a useful tool to assay the therapeutic potential of GSK3 inhibitors and due to the conditional design, it could be used to show that the phenotype produced by excess GSK3 activity is susceptible to reversion when normal GSK3 activity is restored [20].

More recently, mice with conditional and neuronal expression of a dominant negative form of GSK3 (DN-GSK3) have been generated as a tool to predict the neurological consequences of sustained GSK3 inhibition, either *per se* or in combination with mouse models of disease. These mice suffer increased apoptosis in certain brain regions such as the striatum [32] and accordingly, also develop subtle motor abnormalities. This data might predict unwanted side-effects of chronic treatment with GSK3 inhibitors. Fortunately, both the levels of apoptosis and the deficit in motor performance returned to normal upon restoring basal GSK3 activity secondary to transgene silencing [32].

Lithium is a GSK3 inhibitor that is the mainstay for treatment and prophylaxis of bipolar disorder. This compound has also been proposed as a treatment for AD and other neurodegenerative diseases [33], although clinical trials are hampered by lithium's prominent side-effects in the elderly [34]. Since the neurological side-effects of lithium frequently include motor signs such as tremor [35], the similarities with the phenotype of the DN-GSK3 mice led Drs. Raquel Gómez-Sintes and Lucas to hypothesize that therapeutic levels of lithium could also induce neuronal loss through GSK3 inhibition. In fact, they found an induction of neuronal apoptosis in various brain regions associated with the motor deficits in mice treated chronically with lithium [36]. They also found that GSK3 inhibition augmented the translocation of nuclear factor of activated T cells c3/4 (NFATc3/4) transcription factors to the nucleus, leading to increased Fas ligand (FasL) expression and Fas activation. Lithium-induced apoptosis and motor deficits were absent when nuclear translocation of NFAT was prevented by cyclosporin-A administration and in Fas-deficient lpr mice [36]. These results provide evidence of the mechanism by which lithium-induced neuronal and motor toxicity. Moreover, these findings may enable the development of combined therapies that diminish the toxicity of lithium and possibly of other GSK3 inhibitors, extending their potential to treat AD and other neurodegenerative conditions.

The laboratory of Dr. Lucas has also explored the potential contribution of the ubiquitin proteasome system (UPS) in the pathogenesis of neurodegenerative disorders, with special emphasis on Huntington's disease. The experimental approaches employed range from the analysis of the endoproteolytic activities of the proteasome *in vitro* [37,38], or in homogenates from cell and mouse models [39,40], to the use of UPS-reporter transgenic mice [41,42].

SYNAPTIC SIGNALING IN ALZHEIMER'S DISEASE AND THERAPEUTIC OPPORTUNITIES

J. A. Esteban

Summary

AD is a neurodegenerative disorder in which progressive memory loss is accompanied by cognitive decline. AD is of special interest to neuroscientists not only because it is the most common cause of dementia in adults but also because it usually begins with a

remarkably genuine impairment of cognitive function. Despite intensive research, the mechanisms underlying cognitive impairment in AD patients are still not entirely understood. In recent years, a novel hypothesis emerged that subtle alterations in synaptic function might be responsible for the cognitive deficits observed in the initial stages of AD [43,44]. In fact, there is considerable experimental evidence indicating that Aβ alters the mechanisms underlying synaptic plasticity [45,46]. Within this framework, this research group focuses its attention on elucidating the synaptic pathways underlying Aβ-induced dysfunction. In addition, efforts are made to manipulate these pathways experimentally as a therapeutic approach to improve cognitive function.

Synaptic plasticity and its relation to Alzheimer's disease

Synaptic plasticity is the ability of neurons to modify their synaptic connections in response to specific patterns of activation. These modifications lead to long-lasting changes in synaptic efficacy, which are considered as the cellular basis for learning and memory [47]. Two of the best characterized forms of synaptic plasticity in the hippocampus are long-term potentiation (LTP) and long-term depression (LTD), although the precise mechanisms responsible for these forms of synaptic plasticity are still to be elucidated [48]. However, it is well established that the activity-dependent transport of AMPA-type glutamate receptors into and out of excitatory synapses is a critical factor for long-lasting changes in synaptic strength (see Fig. 4) [49].

As mentioned above, Aβ is now thought to contribute to cognitive decline by interfering with synaptic plasticity. Although the precise targets of Aβ are far from clear, it was recently demonstrated that exposure to Aβ oligomers leads to the removal of AMPA receptors from synapses [50], as well as a shift in the balance in synaptic plasticity, impairing LTP and promoting LTD (see Fig. 4) [51–53]. These results are very exciting, since they open the possibility of targeting the mechanisms underlying synaptic plasticity as a therapeutic strategy to correct the synaptic and cognitive dysfunction associated to AD.

Targeting the PIP3 pathway to control Aβ-induced synaptic dysfunction

Multiple signaling pathways appear to mediate these forms of synaptic plasticity, including the PKC, CaMKII, MAPK, and PIP3 pathways [48]. The PIP3 signaling cascade is particularly relevant, since it is

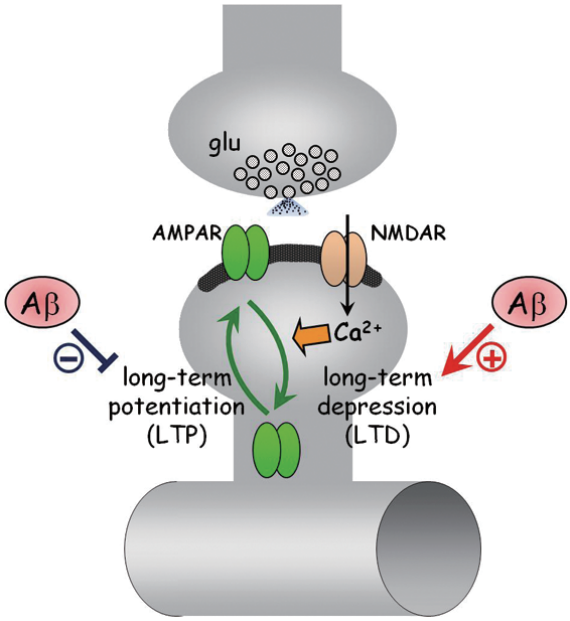

Fig. 4. Model for AMPA receptor trafficking during synaptic plasticity and the potential effects of Aβ. Calcium entry into the postsynaptic terminal upon NMDA receptor activation leads to the delivery of new AMPA receptors to the synaptic membrane (LTP) or to their removal (LTD). Aβ appears to modulate these forms of plasticity in opposite directions, impairing LTP and enhancing LTD.

critically involved in AMPA receptor function [54]. Indeed, deregulation of its downstream effector, GSK3β, appears to be a key feature in AD pathogenesis (see above).

This group has recently presented results indicating that the equilibrium between PI3K and PTEN activity is critical to maintain synaptic function and for the balance in synaptic plasticity, with PI3K favoring synaptic potentiation [54] while PTEN favors synaptic depression (unpublished results). As mentioned above, it is increasingly evident that Aβ disrupts synaptic function by shifting the balance in plasticity towards depression. Therefore, we believe that experimental manipulation of the PI3K-PTEN equilibrium may serve as a therapeutic approach to correct the synaptic dysfunction associated to AD.

Current research in the laboratory is aimed at testing this hypothesis *in vitro* and *in vivo*. To this end, a multidisciplinary approach is being employed that combines an analysis of clinical postmortem samples from patients with AD, molecular biology and electrophysiological assays on brain tissue, and behavioral analyses of mouse models of AD following pharmacological treatment. We believe that this strategy should help understand the basic synaptic alterations underly-

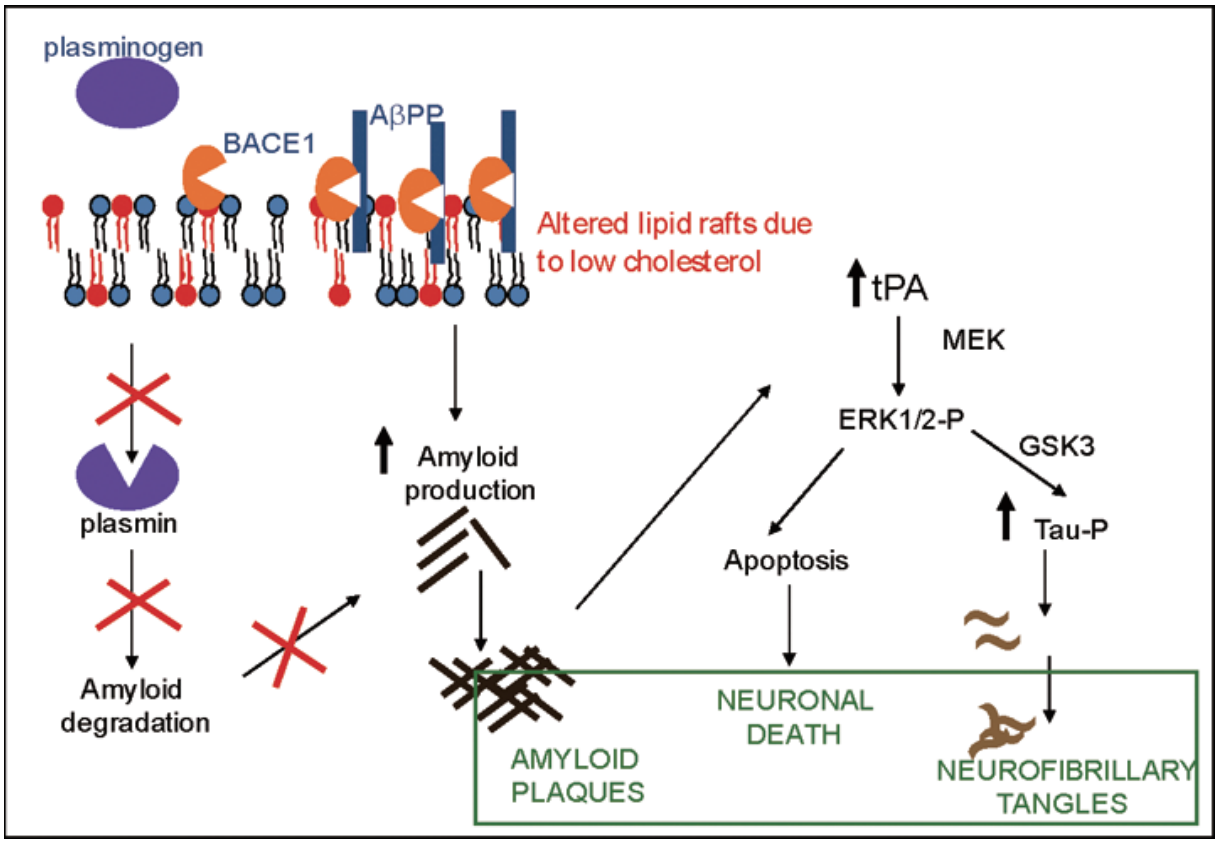

Fig. 5. AD related effects of cholesterol loss. The figure shows one of the working models used by Dr. Ledesma's group to explain the main hallmarks of AD: amyloid accumulation in plaques and tau phosphorylation leading to the generation of neurofibrillary tangles and neuronal death. Based on previous work from the laboratory, this model proposes that the moderate loss of cholesterol detected in the neuronal membrane of AD patients will disrupt lipid raft membrane domains [55]. In turn, these rafts are responsible for the segregation of AβPP and its β-secretase, BACE [62], and for the binding and activation of plasminogen [63]. The disorganization of rafts leads to enhanced contact between AβPP and BACE [62], and to impaired plasmin activation, causing amyloid peptide accumulation [58]. The amyloid peptide induces the production of tissue plasminogen activator, tPA, which in turn activates a cascade of kinases (MEK, ERK1/2, GSK3) leading to tau hyperphosphorylation and neuronal death [63].

ing AD, and at the same time, provide information to advance towards developing new therapeutic approaches to ameliorate the cognitive impairments associated with this devastating disease.

UNDERSTANDING THE ROLE OF LIPIDS IN ALZHEIMER'S DISEASE

M.D. Ledesma

The group led by Dr. Ledesma focuses its research on the role that lipids may play in AD pathology. The data emanating from this and other groups have revealed that the levels of brain cholesterol decrease and those of sphingomyelin increase during aging, as well as in AD patients [55–57]. The question arises as to whether these changes are a cause or a consequence of the disease. To answer this question Dr. Ledesma's group uses two mouse models in which neuronal cholesterol levels are reduced or those of sphingomyelin are increased [58,59] as a result of genetic alteration of the enzymes that control their metabolism [60,61]. These studies aim to determine the influence of these lipid changes on the hallmarks alterations in AD, namely: i) amyloid peptide accumulation (i.e., AβPP processing and plasmin-dependent amyloid degradation); ii) tau phosphorylation; iii) synaptic alterations; iv) oxidative stress; and v) neuronal death. The results obtained to date point to lipid anomalies as having a causal effect in the disease. They also make these mice a suitable experimental model to study brain aging and AD, which will be useful for the preclinical testing of therapeutic strategies based on lipid modulation.

Research is also being carried out to further develop the concepts indicated in the patent application filed by Dr. Ledesma, together with Dr. C.G. Dotti on the "Methods and compositions for treatment of AD by enhancing plasmin or plasmin-like activity" (U.S. Patent Application Serial No. 09/502,448). This opens the group's activities to industrial collaborations and with experts in the plasminogen system, such as the laboratory of Dr. P. Navarro (Instituto Municipal de Investigaciones Medicas – IMIM, Barcelona, Spain). Moreover, the laboratory actively collaborates with groups working on AD, lipid metabolism, or synapse function at the KU Leuven (Belgium), such as those of Drs. C. Bagni, P.P. Van Veldhoven, and C.G. Dotti, as well as with groups at the University of Turin (Italy: Drs. M. Sassoe Pogneto, M. Giustetto, and A. Vercelli). Internal collaborations with the groups working on AD and other neurodegenerative disorders at the CBMSO are assured through weekly meetings to discuss progress in the area.

The group also places an important emphasis on training and there are currently two PhD students working on the aforementioned issues. They are active in disseminating the results through national and international scientific congresses, and in internal and external seminars, which adds to the group's efforts to attract and train scientists who will further develop their research interests in AD in the future.

ALZHEIMER'S DISEASE, THE MOST PROMINENT TAUOPATHY

J. Avila

The group of Dr. Jesús Avila has been working for sometime on the possible roles of the microtubule associated protein tau in AD. In the 1980s, this group found that tau protein can assemble *in vitro* forming PHF with a similar morphology to that found in patient's brain. As a result, several issues related to the phosphorylation and aggregation of tau proteins have been analyzed.

With respect to tau phosphorylation, the protein kinase GSK3 was found to modify the sites identified in phosphorylated tau isolated from the brain of AD patients and, in collaboration with the groups of Drs. Lucas and Hernández at our Center (see above), a transgenic mouse with conditional forebrain overexpression of GSK3β was generated and characterized to analyze, among other issues, tau phosphorylation. Other transgenic mice overexpressing tau were also generated and their characterization is still ongoing. Some of these animals can be used as potential models of the dentate gyrus dysfunction that could take place in AD and that results in the loss of memory in patients.

Another objective of the group is to study how the tau pathology spreads from the hippocampal to the cerebral cortex during AD. At the early stages of this disease, degeneration of neurons occurs in the enthorhinal/hippocampal region and from there the disease spreads to the surrounding areas until it reaches the cortex. The working hypothesis we have adopted to study the spreading of the tau pathology in AD (and perhaps in other tauopathies) was that after the death of the neurons, their cytoplasmic proteins are released into the extracellular space. Some of the proteins released could become toxic in that milieu and the hypothesis is that tau itself could be one such toxic extracellular protein. The initial data that has been obtained appears to support this hypothesis.

In several studies, Dr. Avila's group has collaborated with other groups in Spain, Europe, and USA. In Spain, collaborations are maintained with the groups of Drs. García Verdugo, Ferrer, and Soriano. The group has also collaborated with the group of Dr. Engel (Ireland), and with that of Drs. Perry (Texas), Smith (Ohio), and Vitek (North Carolina) in the USA.

Educational activities

Each week the Neurodegeneration groups hold a seminar (the "neurodegeneration club") to discuss the latest developments in the field.

ACKNOWLEDGMENTS

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